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Journal of Chromatography A, 687 (1994) 303–313

JOURNAL OF CHROMATOGRAPHY A

Confirmational analysis of polycyclic aromatic hydrocarbons in soil extracts by cryotrapping gas chromatography–Fourier transform infrared spectrometry

T. Visser*, M.J. Vredenbregt, A.P.J.M. de Jong

Laboratory for Organic Analytical Chemistry, National Institute for Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, Netherlands

First received 27 June 1994; revised manuscript received 30 August 1994

Abstract

The utility of cryotrapping gas chromatography–Fourier transform infrared spectrometry for isomer differentiation and identification of polycyclic aromatic hydrocarbons (PAHs) in soil samples was investigated. Extracts of sediment, soil and river clay were examined and compared with the results from previous analysis by high-performance liquid chromatography with fluorescence detection. The presence of most of the detected PAHs could be confirmed at a level of 1–4 ng per component injected. In addition, previously undetermined PAHs were identified and valuable structural information was obtained on the identity of two co-eluting isomers. Most of the cryotrapping spectra of PAHs appeared to be similar to spectra recorded with the KBr pelleting technique. Small intensity differences were observed but absorption frequencies were found within the data point resolution of the recorded spectra.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are important environmental pollutants because of the suspected biological activity of most of these compounds and their widespread occurrence. Over the years, a variety of techniques have been developed to determine PAHs both qualitatively and quantitatively. At present, gas chromatography (GC) with flame ionization detection is usually the method of choice. Next, GC combined with mass spectrometry and high-performance liquid chromatography (HPLC) with UV or fluorescence detection are applied when more specific detection of PAH congeners is required. Discrimination of structural isomers however, remains difficult. Unambiguous identification is important as the mutagenity and carcinogenity among the isomers may differ considerably [1]. Additional, complementary information is therefore frequently required.

Infrared spectrometry is known to be very suitable for the discrimination and identification of molecules and for that reason GC combined with Fourier transform infrared spectrometry (GC–FT-IR) can be a helpful tool for the assignment of PAH isomers [2–8] and related compounds such as nitrated PAHs [9], chlorinated biphenyls [10,11] and dibenzodioxins and furans [12–15]. GC–FT-IR analysis of PAHs in real

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00851-5

samples is only rarely reported, mainly because of the limited sensitivity of systems equipped with light-pipe interfacing [2,3,5,7]. Improved detection limits in the (sub)nanogram range are achieved with matrix isolation [4] and cryotrapping or direct-deposition GC–FT-IR [8,16]. These techniques utilize low-temperature storage of the GC eluate to extend the time available for FT–IR analysis, yielding considerably higher sensitivity than is possible with conventional light-pipe interfaces.

The object of this study was to explore the usefulness of cryotrapping GC–FT-IR for the identification of PAHs in complex environmental samples and in particular to confirm the qualitative results from a standard HPLC–fluorescence method, currently used in our laboratory, to determine sixteen so-called "EPA" PAHs. For this purpose, extracts of sediment, soil and river clay were examined in parallel by both techniques.

2. Experimental

2.1. Samples

The sediment, soil and river clay samples were prepared by extraction of 20 g of material with 2×75 mL of light petroleum–acetone (3:1). Clean-up of the extract was performed with a column filled with deactivated aluminium oxide. Sodium sulphate was used as drying agent. The purified extracts were gently dried at room temperature with a flow of nitrogen. The residues were subsequently dissolved in 1 ml of acetonitrile for HPLC analysis. The river clay extract was additionally concentrated to 300 μl for GC–FT-IR measurement.

2.2. HPLC

Reversed-phase HPLC was carried out according to a procedure routinely used in the laboratory. The standard mixture used in this procedure was Standard Reference Material (SRM) 1647b from the National Institute of Standards and Technology (NIST), obtained from C.N. Schmidt (Amsterdam, Netherlands), consisting of sixteen PAHs in acetonitrile solution. The certified concentrations are in the range 3–20 ng/μL.

Separation was performed on an LC 250 pump (Perkin–Elmer) equipped with an automatic WISP injector (Waters). The column, 100×3 mm I.D. (Chrompack), was packed with 5-μm Chromspher PAH. The column temperature was maintained at 30°C. Gradient elution was executed with acetonitrile–water (40:60)(A) and acetonitrile (B). The gradient programme was 0–2 min, 90% A–10% B; 2–27 min, linear change to 0% A–100% B; 27–32 min, 0% A–100% B; 32–34 min, linear change to 90% A–10% B and 34–39 min, 90% A–10% B. The flow-rate was 1 ml/min and the volume injected was 10 μl.

2.3. Fluorescence detection

A Waters FLU 470 spectrometer was used for fluorescence detection. The excitation/emission wavelengths were as follows: 0–11.5 min, 253/333 nm; 11.5–14.2 min, 253/373 nm; 14.2–17.5 min, 263/420 nm; 17.5–22.5 min, 270/382 nm; and 22.5–39 min, 280/460 nm. The internal standard was 6 methylchrysene. Peak heights were used for quantification.

2.4. Gas chromatography

GC separations were performed on a Carlo Erba MEGA 5160 gas chromatograph using a split–splitless injector. The gas chromatograph was equipped with a DB-17 capillary column (J&W Scientific) (15 m $\times$ 0.25 mm I.D., film thickness 0.15 μm). This column was chosen because it gave a good separation between the isomers of fluoranthene and between benzo[*a*]pyrene and benzo[*e*] pyrene. In addition, thin-film stationary phases are preferred for cryotrapping GC–FT-IR because of the lower column bleeding compared with thick-film columns. According to Smyrl et al. [8], column bleeding may cause severe deterioration of the GC–FT-IR performance.

The injector temperature was 250°C and the

injection volume was 1 μl. The carrier gas was helium with a calculated flow-rate of 0.8 ml/min. After optimization the following temperature programme was chosen for the analysis of the extracts: 60°C, for 3 min, followed by an increase of 20°C/min to 140°C and then 5°C/min to 290°C, which was held isothermal for 10 min.

SRM 2260 (obtained from C.N. Schmidt) NIST, consisting of 24 PAHs in toluene solution, was used for chromatographic optimization and to establish retention times and detection limits. The nominal concentration of the PAHs in the standard solution was 60 ng/μl. Aliquots of 200 μl were diluted to concentrations of 30, 2, 1, 0.5 and 0.25 ng/μl. Standard solutions of 60 and 30 ng/μl were injected with splitting ratios of 1 : 10 and 1 : 30. Standards with concentrations of 2, 1, 0.5 and 0.25 ng/μl and all extracts were analysed with splitless injection.

2.5. FT-IR spectrometry

Infrared spectrometric detection was carried out with a Digilab (Biorad) (FTS-40 Fourier transform instrument equipped with a Digilab Tracer cryotrapping GC interface and an SPC 3200 computer for data acquisition and processing. A detailed description of the interface is given in Ref. [8]. Connection between the GC column and the FT-IR interface was accomplished with a 1-m deactivated fused-silica transfer line of 150 μm I.D. by means of a silver ferrule connector (Bio-rad). A fused-silica deposition tip of 150 μm I.D. was fixed at the end of the transfer line, acting as a restricting element to reduce the operating flow-rate to ca. 1 ml/min. The transfer line and deposition tip were heated to 290°C. The tip was located 30 μm above the surface of a moving ZnSe window, which was cooled with liquid nitrogen to 77 K. Stepwise shifting of the window was accomplished with an X–Y stepper motor. The standard software programme TRTP was used to reduce the number of steps per second at proceeding retention time in order to compensate for GC peak broadening. The high-vacuum interface housing was held at 10^{-5} Torr to prevent condensation of atmospheric substances.

Spectra of the trapped GC eluate were recorded "on-the-fly", i.e., a few seconds after deposition, by averaging four scans every 2 s with an optical resolution of 8 cm^{-1}. Incidentally, post-run scanning of trapped components was performed after completion of the GC run by repositioning the corresponding window coordinates (i.e. retention times) into the IR beam. Post-run spectra were recorded with 512 scans co-added and an optical resolution of 2 or 8 cm^{-1}.

GC–FT-IR traces of integrated IR absorption as a function of time were constructed by standard Gram–Schmidt vector orthogonalization. A so-called functional group (Fg) chromatogram of the preselected wavenumber region 700–950 cm^{-1} was used for monitoring the elution of PAHs as the strongest absorptions of these compounds are found in this region. In addition, interference of eluting non-aromatics is relatively small in this wavenumber interval.

3. Results and discussion

The functional group GC–FT-IR trace at 700–950 cm^{-1} of a standard solution of SRM 2260 is shown in Fig. 1. The identity of all 24 PAH components was established from the elution sequence combined with the similarity between the obtained cryotrapping spectra and the KBr reference spectra from the literature [17,18] or

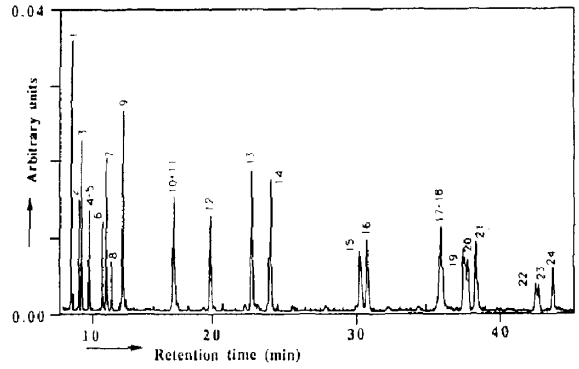

Fig. 1. Functional group GC–FT-IR (700–950 cm^{-1}) of the standard solution of SRM 2260. Concentration 60 ng/μl per component; splitting ratio 1 : 10.

from an in-house recorded collection. The corresponding retention times (t_R) and concentrations are summarized in Table 1. The reproducibility of t_R was established from three subsequent injections to be within 0.03 min.

Under the chromatographic conditions used, not all compounds were uniquely separated. However, interference-free spectra of all components were obtained either straightforwardly from individual chromatographic peaks or from spectra on the wings of a GC peak in case of co-elution.

All 24 cryotrapping FT-IR spectra were found to differ, evidently endorsing the discriminative properties of FT-IR. Typical examples are the spectra of the isomers benzo[*a*]pyrene and benzo[*e*]pyrene as shown in Fig. 2. In addition to the relatively strong absorption bands of the =C–H out-of-plane bending vibrations in the region 700–950 cm^{-1}, the bands in the in-plane =C–H and skeletal region 1600–1000 cm^{-1} appeared to be useful for distinguishing between the different compounds and isomers.

Differences between the cryotrapping IR spectra of the 24 PAHs and the spectra recorded with the conventional KBr pelleting technique appeared to be small. The intensities of the absorption bands may differ slightly, but the band maxima are within the data point resolution (4 cm^{-1}) of the on-the-fly spectra. This is in agreement with earlier findings [8,16] and it endorses that reference collections of conventionally recorded spectra can be helpful for identification purposes. Band narrowing as an effect of the low temperature (77 K) as reported by Smyrl et al. [8] occurred only to a negligible extent in the

Table 1
Identity, retention times (t_R) and concentrations of PAHs in SRM 2260

Peak No.[a]	Component	t_R (min)	Concentration (ng/μl)
1	Naphthalene	7.51	66.0
2	2-Methylnaphthalene	8.51	65.5
3	1-Methylnaphthalene	8.91	65.5
4	Biphenyl	9.67	65.8
5	2,6-Dimethylnaphthalene	9.77	65.6
6	Acenaphthylene	11.20	63.2
7	Acenaphthene	11.57	68.2
8	2,3,5-Trimethylnaphthalene	12.12	58.4
9	Fluorene	13.12	65.4
10	Phenanthrene	17.29	65.7
11	Anthracene	17.31	49.7
12	1-Methylphenanthrene	20.01	65.0
13	Fluoranthene	22.88	66.0
14	Pyrene	24.15	66.0
15	Benz[*a*]anthracene	30.04	57.1
16	Chrysene	30.43	66.2
17	Benzo[*b*]fluoranthene	35.18	65.7
18	Benzo[*k*]fluoranthene	35.35	65.4
19	Benzo[*e*]pyrene	36.85	65.7
20	Benzo[*a*]pyrene	37.10	59.3
21	Perylene	37.71	49.7
22	Indeno[1,2,3-cd]pyrene	42.67	58.3
23	Dibenz[*a,h*]anthracene	42.98	49.3
24	Benzo[*ghi*]perylene	44.91	58.7

[a] Peak numbers refer to Fig. 1.

Table 2
Comparison of HPLC–fluorescence and GC–FT-IR analysis of PAHs in a sediment sample

Peak No.[a]	Component	HPLC detected[b]	GC–FT-IR identified[b]
9	Fluorene	+	–
10	Phenanthrene	+	+
11	Anthracene	+	+
13	Fluoranthene	+	+
14	Pyrene	+	+
15	Benz[*a*]anthracene	+	+
16	Chrysene	+	+
17	Benzo[*b*]fluoranthene	+	+
18	Benzo[*k*]fluoranthene	+	–
19	Benzo[*e*]pyrene	–	+
20	Benzo[*a*]pyrene	+	+
21	Perylene	–	+
22	Indeno[1,2,3-*cd*]pyrene	+	+
23	Dibenz[*a*,*h*]anthracene	+	–
24	Benzo[*ghi*]perylene	+	+

[a] Peak numbers refer to Fig. 3.
[b] + Positively detected or confirmed; – not detected or confirmed.

Table 3
Comparison of HPLC–fluorescence and GC–FT-IR analysis of PAHs in a soil sample

Peak No.[a]	Component	HPLC detected[b]	GC–FT-IR identified[b]
9	Fluorene	+	–
10	Phenanthrene	+	+
11	Anthracene	+	–
12	1-Methylphenanthene	–	+
13	Fluoranthene	+	+
14	Pyrene	+	+
15	Benz[*a*]anthracene	+	–
16	Chrysene	+	–
17	Benzo[*b*]fluoranthene	+	+
◆	Benzo[*j*]fluoranthene	–	+
18	Benzo[*k*]fluoranthene	+	–
19	Benzo[*e*]pyrene	–	+
20	Benzo[*a*]pyrene	+	–
22	Indeno[1,2,3-*cd*]pyrene	+	+
23	Dibenz[*a*,*h*]anthracene	+	–
24	Benzo[*ghi*]perylene	+	–

[a] Peak numbers refer to Fig. 4.
[b] + Positively detected or confirmed; – not detected or confirmed.

Table 4
Comparison of HPLC–fluorescence and GC–FT-IR analysis of PAHs in a river clay sample

Peak No.[a]	Component	HPLC detected[b]	GC–FT-IR identified[b]
10	Phenanthrene	+	+
11	Anthracene	+	–
13	Fluoranthene	+	+
14	Pyrene	+	+
15	Benz[*a*]anthracene	+	+
16	Chrysene	+	+
17	Benzo[*b*]fluoranthene	+	+
18	Benzo[*k*]fluoranthene	+	+
19	Benzo[*e*]pyrene	–	+
20	Benzo[*a*]pyrene	+	–
22	Indeno[1,2,3-*cd*]pyrene	+	–
23	Dibenz[*a,h*]anthracene	+	–
24	Benzo[*ghi*]perylene	+	–

[a] Peak numbers refer to Fig. 5.
[b] + Positively detected or confirmed; – not detected or confirmed.

postrun spectra of the volatile naphthalenes and biphenyl, recorded with a data point resolution of 1 cm^{-1}.

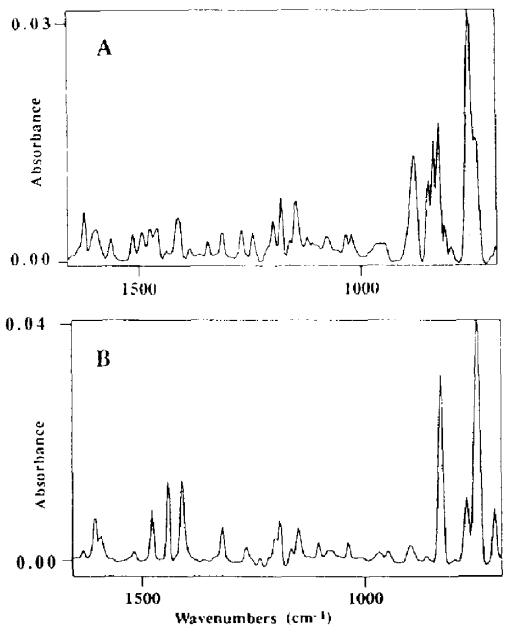

Fig. 2. Cryotrapping GC–FT-IR spectra of the isomers (A) benzo[*a*]pyrene and (B) benzo[*e*]pyrene taken from the chromatogram in Fig. 1.

3.1. Detection limits

Although the aim of this study was to confirm results of HPLC–fluorescence analysis qualitatively, experiments with dilute standard solutions were carried out to obtain an insight into the sensitivity of GC–FT-IR for PAH analysis. Elution of all components of the dilute standards was seen in the Fg chromatogram at 950–700 cm^{-1} to a smallest amount of 1 ng injected. Amounts of 0.5 ng were no longer visible in the chromatograms but identifiable spectra were obtained by carrying out postrun measurements at the previously established retention time window (see Table 1). Postrun measurement of the 0.25-ng injections only provided spectra of naphthalene, fluorene, fluoranthene and pyrene.

3.2. Samples

GC–FT-IR analysis of soil extracts in acetonitrile was hampered by a limitation in the deposition mechanism of the GC–FT-IR interface. When using splitless injection, the large amount of solvent causes a vapour cloud at the end of the deposition tip which is partly spread over the deposition window. Despite the high vacuum in the GC–FT-IR interface, solvent crystallization

on the window occurs, usually in an area that covers the point where immobilization of the chromatogram begins. As result of this effect, the first 10–15 min of the GC trace of samples in acetonitrile were found to be IR opaque and no information could be obtained in this region. Therefore, PAHs with $t_R \leq 15$ min were not taken into account.

Results of the comparative GC–FT-IR and HPLC–fluorescence determinations of PAHs in sediment, soil and river clay extracts are summarized in Tables 2, 3 and 4, respectively. The corresponding chromatograms are shown in Figs. 3, 4 and 5. As can be seen, the signal-to-noise ratio of the GC–FT-IR traces is much lower than that of the HPLC–fluorescence traces, owing to the lower sensitivity of FT-IR. In addition, the chemical background is higher as fluorescence detection is transparent to most of the non-PAH compounds, whereas, inherent to the FT-IR principle, the FT-IR chromatogram comprises full-spectrum data of all eluting substances. As a

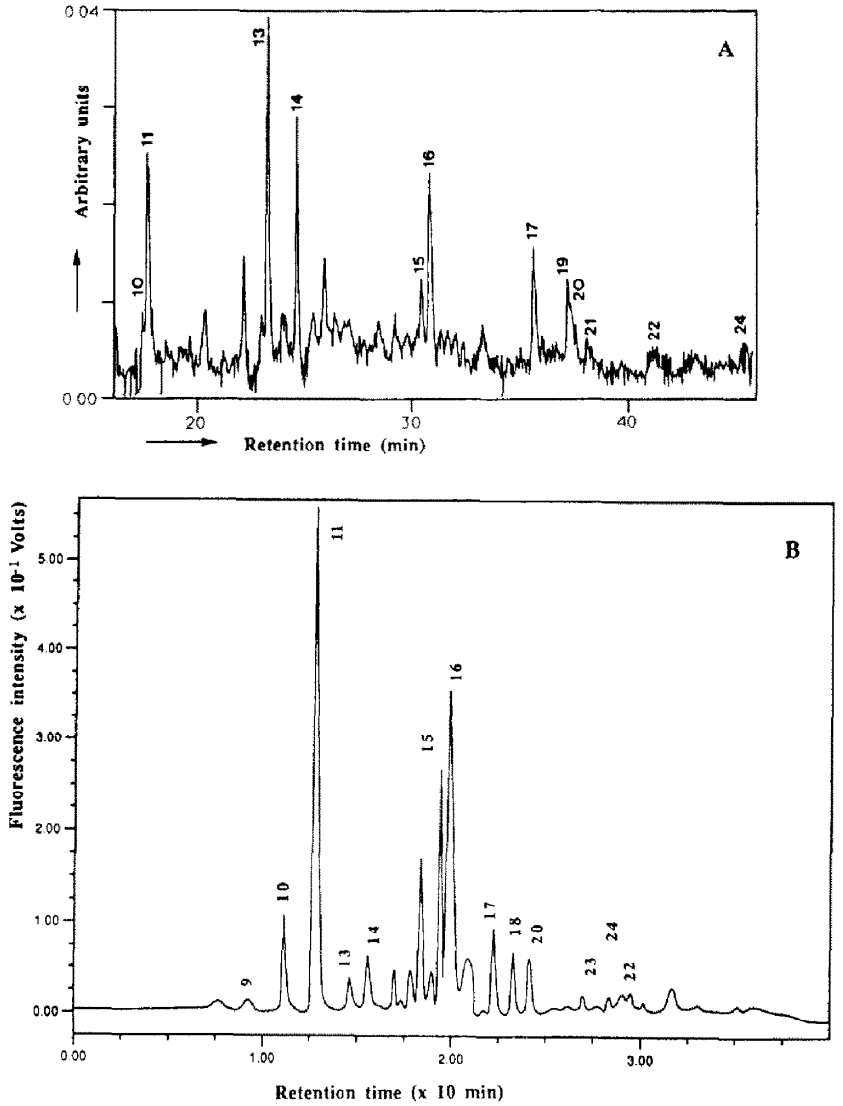

Fig. 3. (A) Functional group GC–FT-IR (A) and (B) HPLC–fluorescence analysis of a sediment extract.

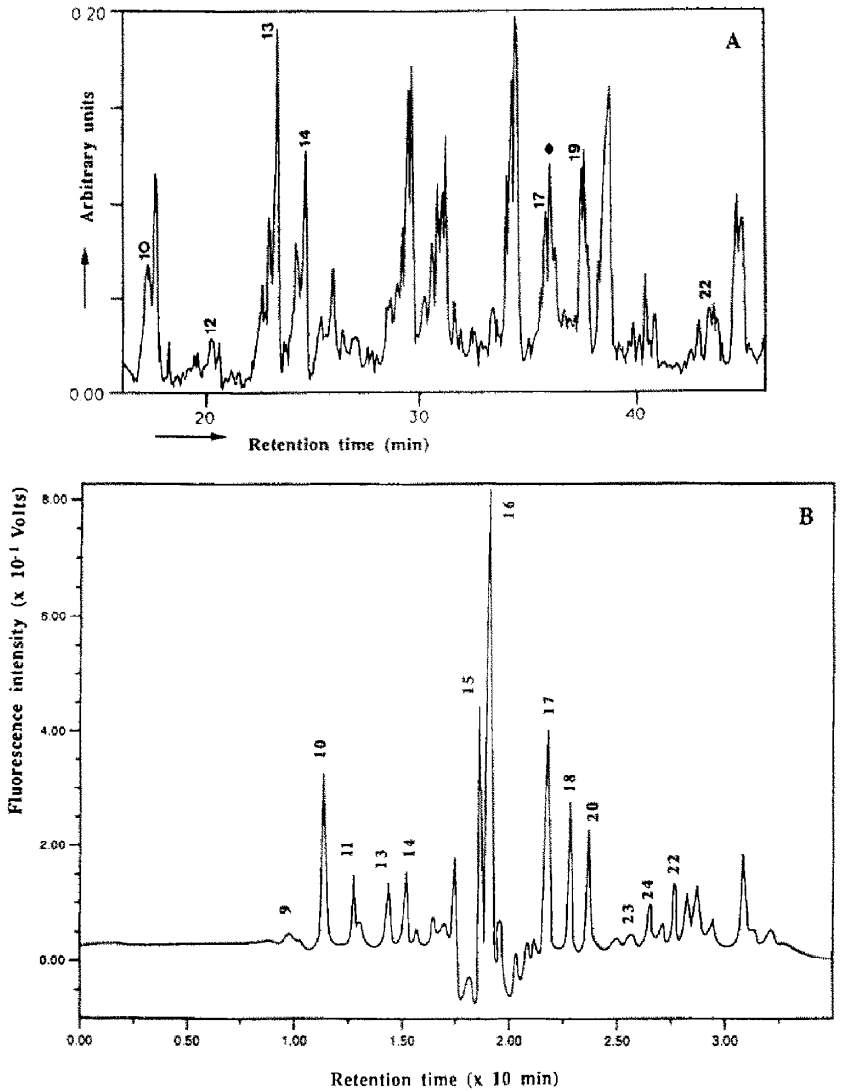

Fig. 4. (A) Functional group GC–FT-IR (A) and (B) HPLC–fluorescence analysis of a soil extract.

consequence, IR spectra may contain interfering absorption bands of co-eluting compounds. Incidentally, this phenomenon was observed. In the sediment extract for instance, the spectrum shown in Fig. 6 was obtained at a retention time matching with that of the benzo[*e*]pyrene standard. The molecular fingerprint region clearly shows all significant bands present in the reference spectrum of this compound (Fig. 2B) and therefore it has been identified positively, despite the presence of absorptions at 1450 and 1020 cm^{-1}. Matching of retention times and agreement in IR absorption frequencies of at least five characteristic bands in the sample and reference spectra turned out to be a useful criterion for the positive identification of this type of compound and, following this procedure, the occurrence of twelve PAH was confirmed in the sediment sample.

Compared with the results of the HPLC analysis, the presence of benzo[*k*]fluoranthene and dibenz[*a*,*h*]anthracene could not be confirmed in

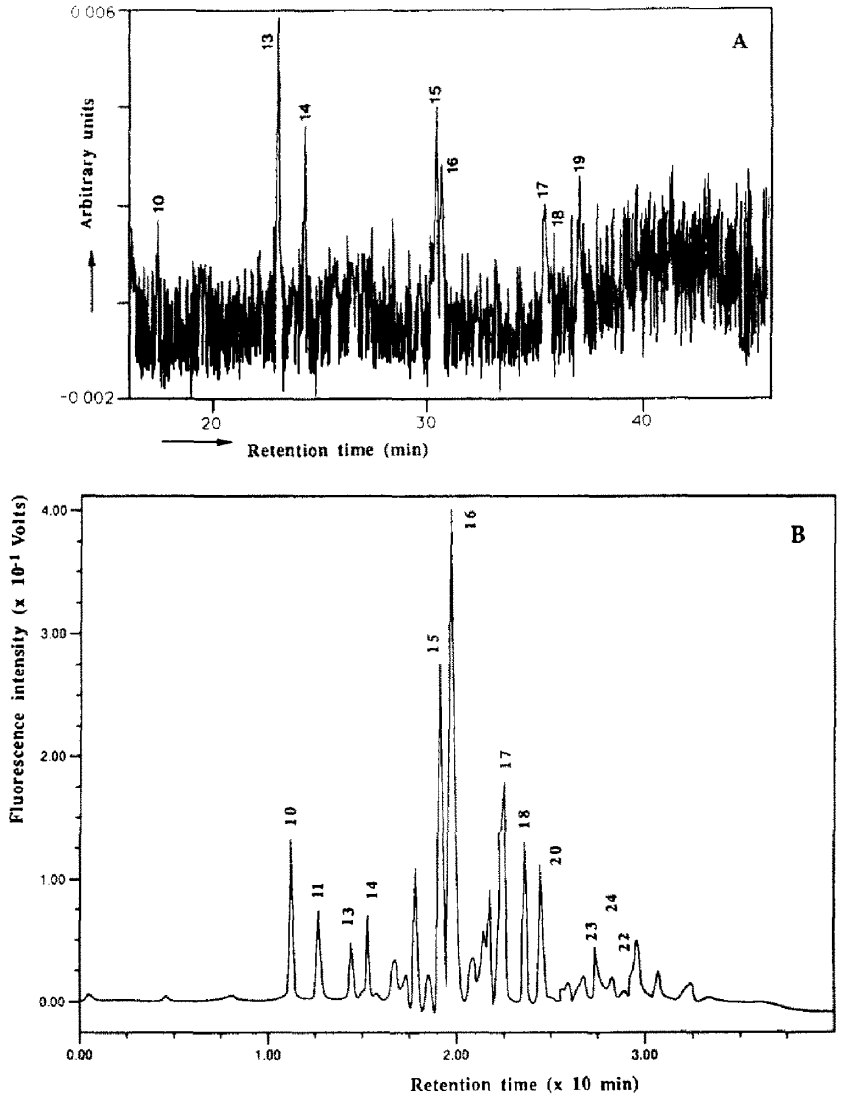

Fig. 5. (A) Functional group GC–FT-IR and (B) HPLC–fluorescence analysis of a river clay extract.

the sediment extract because of insufficient sensitivity of the GC–FT-IR system. In contrast, perylene, benzo[*e*]pyrene and related (poly) aromatic compounds such as anthraquinone have been identified. Indeed, these compounds were not determined by HPLC–fluorescence analysis, but it should be noted that these components were absent in the HPLC reference standard.

The high chemical background in the GC–FT-IR trace of the soil extract (Fig. 4A) hampered identification of several PAHs, determined by HPLC–fluorescence. Eight compounds could be identified by precise selection of background spectra in the close vicinity of the chromatographic peak of interest. Seven of these were determined directly with the help of the IR spectra obtained from the SRM 2260 solution. Benzo[*j*]fluoranthene was not identified straightforwardly as it was absent in the standards. However, a library search in a reference collection of KBr spectra revealed a high similarity index for the spectrum of this molecule. The

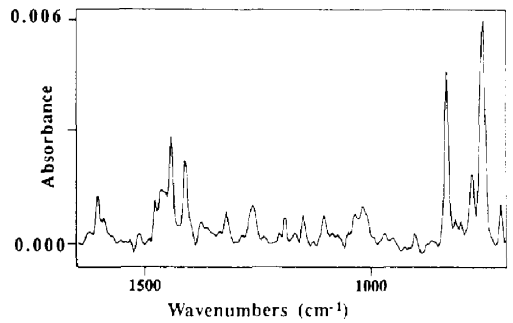

Fig. 6. Cryotrapping spectrum of benzo[*e*]pyrene with interfering bands at 1450 and 1020 cm^{-1}, obtained from peak 19 in the chromatogram in Fig. 3A.

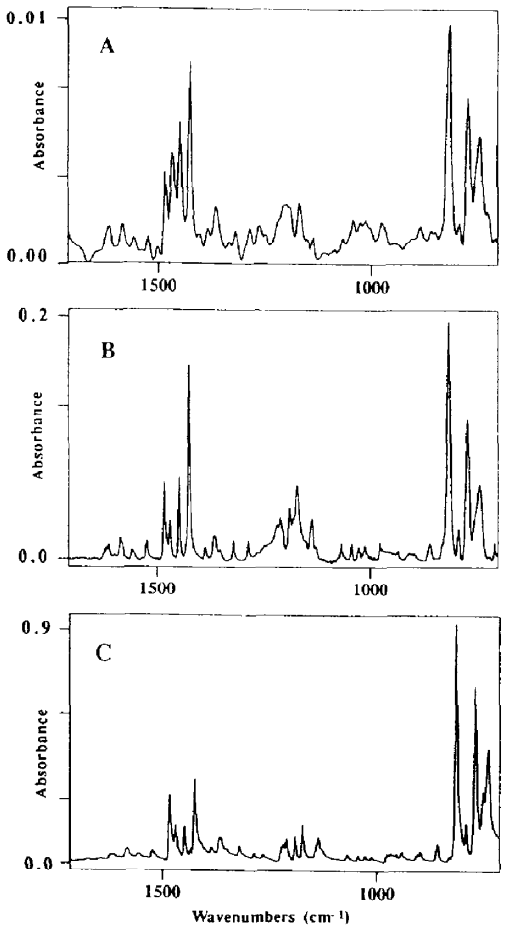

Fig. 7. IR spectra of benzo[*j*]fluoranthene: (A) cryotrapping spectrum obtained from peak ◆ in the chromatogram in Fig. 4A; (B) cryotrapping spectrum obtained from a standard solution; (C) KBr reference spectrum.

identity was checked by separate recording of the cryotrapping spectrum of the standard. All three spectra are shown in Fig. 7.

The signal-to-noise ratio of the GC–FT-IR trace of the river clay extract (Fig. 5A) was very poor as a result of the low concentration of the components (estimated as 0.5–2 ng/μl in the concentrated extract). For that reason, the presence of only eight PAHs could be confirmed.

The discriminative value of FT-IR detection was demonstrated by the identification of benzo[*e*]pyrene in all three extracts. Initially, HPLC analysis indicated the presence of the [*a*] isomer only, but this could not be confirmed with GC–FT-IR. In contrast, the IR spectrum of the [*e*] congener was identified in the sediment, soil and river clay extracts. Closer examination of the HPLC analysis, however, indicated that, under the chosen experimental conditions, co-elution of the [*a*] and [*e*] isomers may occur. Combined with insufficient detector specificity, this probably caused incorrect conclusions.

4. Conclusions

It is concluded that cryotrapping GC–FT-IR can be a helpful tool for the qualitative analysis of PAHs in environmental samples. The results of HPLC–fluorescence analysis of three soil extracts have been largely confirmed. In addition, the presence of other (poly)aromatic compounds was established. The technique appears to be specifically suited to distinguishing between structural isomers, which is particularly valuable in case co-elution in the HPLC analysis occurs.

The detection limit is in the range 0.25–1 ng injected for standard solutions and 1–4 ng for soil extracts. As such, cryotrapping GC–FT-IR can be a valuable confirmational technique for PAHs in addition to GC–FID, GC–MS and HPLC–UV/fluorescence.

It was endorsed that cryotrapping IR spectra of PAHs largely resemble spectra recorded with the KBr pelleting technique at room temperature. Consequently, existing spectrum libraries can be used for the interpretation and identification of cryotrapping spectra of unknowns.

Acknowledgements

Ms. C.J. Berkhoff, Ms. W.C. Hijman and Mr. J.Y. Wammes are grateful acknowledged for the preparation of the extracts and for carrying out the HPLC analyses.

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